METHOD FOR TRANSFORMING EPOXIDES CARRYING TRIFLUOROMETHYL GROUPS

The present invention relates to a process for the hydrolysis of fluorinated epoxides comprising one or more CF_3 groups, and more particularly to a process for treating a mixture of (R) and (S) enantiomers of such fluorinated epoxides, so as to enrich the mixture in one of the enantiomers of this epoxide and to obtain, in parallel, the vicinal diol corresponding to the other enantiomer. It relates in particular to a process for separating the (R) and (S) enantiomers, and more particularly to a process for enriching in isomer of absolute configuration (S) and in diol of configuration (R), or, conversely, enriching in isomer of absolute configuration (R) and in diol of configuration (S). The invention also relates to a process for producing the enantiomers and/or the vicinal diols in enantiopure or enantiomerically enriched form.

Another application of this process is the nonenantioselective biohydrolysis of a racemic or nonracemic epoxide.

Epoxides are intermediates that are very important in organic synthesis because they possess high reactivity. They in fact combine the advantage of possessing a substantial cycle tension and of having a nucleofugal oxygen atom. The presence of an asymmetric carbon results in molecules having two distinct stereoisomeric forms (enantiomers), the (R) form and the (S) form, one being the mirror image of the other. It may be important, in certain cases, to have only one of these forms and it is then advisable to have means for separating these two stereoisomers or for specifically synthesizing the desired stereoisomer.

Fluorinated molecules have an advantageous position both in agrochemical and in pharmacy. Access to fluorinated epoxides and vicinal diols of (R) or (S) configuration is therefore advantageous, in particular as an intermediate for the synthesis of these fluorinated molecules.

The use of microscopic fungi and of proteins of fungal origin has been described as being able to be of use in the separation of these stereoisomers of certain epoxides.

Thus, S. Pedragosa-Moreau et al. (J. Org. Chem. 1995, 61: 7402-7407) describe the use of epoxide hydrolase for the synthesis of enantiopure para-substituted styrene oxide. They neither describe nor suggest application to epoxides carrying CF_3 groups. EP-A-0 611 826 describes a process for producing optically enriched epoxides using microscopic fungi selected from various genera. According to the fungus used, the reaction makes it possible to prepare the (R) form or the (S) form of an epoxide.

WO-A-0068394 describes the isolation, the cloning and the overexpression of an enzyme referred to as "epoxide hydrolase" from a fungus of the *Aspergillus* genus and the use of this epoxide hydrolase for preparing enantiomerically enriched molecules from mixtures of isomers of epoxide compounds described in very full terms. A study carried out on *para*-nitrostyrene oxide has revealed a greater affinity and a greater catalytic constant of the enzyme for the (R) enantiomer compared with the (S) enantiomer, resulting in rapid hydrolysis of the (R) isomer to its corresponding diol. That document discloses the protein sequence and the nucleotide sequence of the epoxide hydrolase of an *Aspergillus niger* fungus, which allows that document to propose the production of the enzyme by genetic engineering.

As recalled in document EP-A-0 611 826, numerous classes of epoxides exist. The process is exemplified in that document with respect to a restricted number of compounds, namely 3-chlorostyrene oxide, glycidol, allyl glycidyl ether, 3,4-epoxy-1-butene, 1,2-epoxy-hexane, 2,3-epoxypropylbenzene and styrene oxide, whereas the variety of epoxides, and of the reaction groups that they are capable of carrying, is very vast.

An epoxide hydrolase activity, and in particular an enantioselective epoxide hydrolase activity, on epoxides comprising one or more CF_3 groups, has never been demonstrated. Given the specific nature of epoxides comprising one or more CF_3 groups, in particular because of the considerable electronegativity of the fluorine, it was notably impossible for those skilled in the art to predict the reactivity and the specificity of a specific enzyme according to the invention relative to this type of substrate.

The main objective of the present invention is therefore to provide a process for the hydrolysis of epoxides carrying trifluoromethyl units.

A subject of the invention is more particularly such a process for separating the (R) and (S) enantiomers of epoxides carrying trifluoromethyl units, from a racemic or nonracemic mixture of enantiomers of said epoxide.

Another objective of the invention is to provide such a process that can be used for preparing epoxides or diols as intermediates for the synthesis of pharmaceutical, agrochemical or plant protection products.

The applicants have been able to demonstrate, for the first time, that epoxides carrying trifluoromethyl

units can be hydrolyzed with opening of the epoxide and formation of a diol, and, in addition, that these epoxides can be separated enantioselectively, using an epoxide hydrolase such as that of *Aspergillus niger* LCP521. This has allowed them to develop a process for the enantioselective conversion of racemic or nonracemic CF_3 epoxides, based on the use of this epoxide hydrolase or of a similar protein or polypeptide having an epoxide hydrolase activity on epoxides comprising a trifluoromethyl unit.

According to a variant of the invention, and as will be seen below, the conversion can be carried out under conditions which are not, or are not very, enantioselective, and advantage can be taken of this variant for a nonenantioselective or not very enantioselective hydrolysis of epoxides.

A subject of the present invention is therefore a process for the hydrolysis of a fluorinated epoxide comprising one or more CF_3 groups, preferably an epoxide of formula (I) as described below, in which process the epoxide is treated, in the presence of water, with a protein having an epoxide hydrolase (EH) activity on CF_3 epoxides so as to induce opening of the epoxide and formation of the vicinal diol. The epoxide can be an (R) or (S) isomer, or a racemic or nonracemic mixture of these isomers.

The subject of the present invention is more particularly a process for converting a mixture of (R) and (S) enantiomers of a fluorinated epoxide comprising one or more CF_3 groups, preferably an epoxide of formula (I), into a mixture enriched in one of the isomers and in the diol corresponding to the other isomer, in which process: (A) a mixture of (R) and (S) enantiomers of the epoxide according to the invention is treated with (B) a protein having an epoxide hydrolase (EH) activity on CF_3 epoxides, the process

resulting in the preferential opening either of the (R) epoxide to form the (R) diol or of the (S) epoxide to form the (S) diol.

By definition, the opening of an epoxide with a water molecule is said to be "preferential" in the sense that the protein has a greater affinity and a greater catalytic constant for one of the enantiomers compared with the other, which is reflected by a greater rate of hydrolysis of this enantiomer. It is therefore possible, by controlling the hydrolysis reaction, in particular by stopping this reaction at the appropriate time, to obtain, at a given instance, a composition enriched in (R) or (S) epoxide and in the diol of opposite absolute configuration.

The process according to the invention generally results in a resolving reaction in which one enantiomer of the epoxide of (R) or (S) configuration is opened so as to give the corresponding diol. The epoxide hydrolase in accordance with the invention can therefore induce an enantioselective hydrolysis of these specific epoxides, in notable and unexpected proportions. The conversion has a particularly advantageous enantioselective nature when the enantioselectivity coefficient (E) (see below) is greater than or equal to 10, preferably greater than or equal to 30. Depending on the epoxide substrate, the EH can preferentially hydrolyze either the (R) isomer or the (S) isomer. It is found that, with the majority of epoxides, the EH preferentially hydrolyzes the (R) isomer.

By convention, in the present disclosure, the terms or expressions "enzyme", "enzyme with epoxide hydrolase activity" and "protein with epoxide hydrolase activity" are synonyms and are used without distinction.

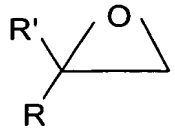
The process can comprise a subsequent step consisting of separation of the (S) epoxide from the (R) diol or, conversely, separation of the (R) epoxide from the (S) diol, so as to recover, firstly, a composition enantiomerically enriched in epoxide and, secondly, a composition enriched in diol. With preferential hydrolysis of the (R) isomer, the separation step results in the recovery of a composition enriched in (S) epoxide and a composition enriched in (R) diol. With preferential hydrolysis of the (S) isomer, the separation step results in the recovery of a composition enriched in (R) epoxide and a composition enriched in (S) diol. It is possible to carry out, as required, at least one other treatment of the composition enriched in epoxide with the enzyme, and then extraction and separation.

The composition enriched in (R) or (S) diol can be subjected to a cyclization of the (R) or (S) diol into the (R) or (S) epoxide, and then, as required, to at least one other treatment with the enzyme, followed by a further cyclization.

The aim of successive (2 or more) treatments is to improve, as required, the enantiomeric excess of the corresponding enantiomer.

Definition of the epoxides

The epoxides targeted by the invention comprise one or more CF_3 groups, preferably from 1 to 3, and may preferably correspond to formula (I):



in which:

- the group R is an alkyl, alkenyl, cycloalkyl, aryl or aralkyl group optionally substituted with alkyl, alkoxy, alkylthio or halogen; R optionally comprising one or more hetero atoms such as O or S; the alkyl, alkoxy and alkylthio substituents comprising a linear, branched or cyclic C_1-C_6, preferably C_1-C_3, hydrocarbon-based chain, optionally comprising one or more halogen atoms, such as Cl, F or Br, preferably F;

- the group R' is H or a linear, branched or cyclic C_1-C_{10}, preferably C_1, C_2 or C_3 alkyl, optionally comprising one or more hetero atoms, in particular halogen atoms, such as Cl, F or Br, preferably F, or else hetero atoms such as O or S;

- it being understood that at least one of the radicals R and R' is, or comprises, one or more, preferably from 1 to 3, trifluoromethyl (CF_3) groups;

with (B) a protein having an epoxide hydrolase (EH) activity on CF_3 epoxides.

According to a preferred embodiment, the epoxide of formula (I) is such that R' is H or a C_1, C_2 or C_3 linear alkyl, or better still R' is H or C_1 alkyl optionally substituted with one or more halogen atoms, preferably F, for example substituted with 3 F atoms.

The groups R can comprise from 1 to 20 C, in particular from 1 to 12 C.

When R is an alkyl substituted with a halogen, R can be CF_3.

The groups R can be substituted with from 1 to 3 groups selected from trifluoromethyl, trifluoromethoxy and trifluoromethylthio.

The alkyl groups R can be linear or branched. They preferably comprise from 1 to 10 C, more preferentially from 1 to 6 C. For example: methyl, propyl, isopropyl, butyl, isobutyl, pentyl, isopentyl, hexyl or isohexyl,

optionally substituted with one or more halogen atoms, such as Cl, F or Br, preferably F. The alkyl group R preferably comprises from 1 to 3 CF_3 groups.

The cycloalkyl groups R preferably comprise from 3 to 10 C, preferably from 3 to 8 C, better still from 5 to 7 C. For example: cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cyclooctyl, optionally substituted with one or more halogen atoms, such as Cl, F or Br, preferably F. The cycloalkyl group R preferably comprises from 1 to 3 CF_3 groups.

The aryl groups R can, for example, be phenyl and naphthyl groups, optionally substituted with one or more halogen atoms, such as Cl, F or Br, preferably F. The aryl group R preferably comprises from 1 to 3 CF_3 groups. The phenyl groups thus substituted are preferred modalities.

The aralkyl groups R can in particular comprise from 7 to 18 C. By way of examples, mention may be made of benzyl, 1-methylbenzyl, 2-phenylethyl, 3-phenylpropyl, 4-phenylbutyl, 1-naphthylmethyl or 2-naphthylmethyl groups, optionally substituted with one or more halogen atoms, such as Cl, F or Br, preferably F. The aralkyl group R preferably comprises from 1 to 3 CF_3 groups.

The epoxides comprising a phenyl radical R comprising a phenyl group bearing from 1 to 3, preferably 1 or 2, CF_3 groups, optionally trifluoromethoxy or trifluoromethylthio, are preferred modalities of the invention. It can also be specified that these groups may be in the para-, ortho- or meta-position with respect to the carbon of the phenyl connected to the oxirane. A substitution in the para- or meta-position is, however, preferred, and even more preferentially in the para-position. These preferred epoxides can also comprise a gem-disubstitution with an R' preferably selected from C_1-C_3 alkyls, preferably CH_3. These epoxides can also be

optionally substituted with one or more halogen atoms, such as Cl, F or Br, preferably F. Several advantageous examples of phenyl oxiranes substituted with CF_3 in the 2-, 3-, 4- and 3,5-positions, or substituted with $-O-CF_3$ or $-S-CF_3$ in the 4-position, R' being H or CH_3, are described in the examples.

The epoxide hydrolase

A protein having an epoxide hydrolase (EH) activity on CF_3 epoxides is represented by the protein having as amino acid sequence the sequence described in SEQ ID NO: 2. This protein is the epoxide hydrolase of *Aspergillus niger* registered with the Natural History Museum (Paris) under the number LCP521 (Laboratory of Cryptogamy, 12 rue Buffon, 75005 Paris, France). This protein has been described in the publication WO-A-00 68394, to which those skilled in the art may refer as required. This protein constitutes, for the needs of the present invention, the reference protein and at the same time constitutes a preferred embodiment.

The protein can also be a "variant", "homolog", or "derivative" of the reference protein, which, by definition, has, like the reference protein, an EH activity on a CF_3 epoxide, and preferably a biological activity that is at least identical, similar or analogous to the reference protein on the same substrate.

In the case of application to an enantioselective hydrolase, the preferred proteins are those that have, for a given substrate, an enantioselectivity coefficient E greater than or equal to 10, preferably greater than or equal to 30, more preferably greater than or equal to 50, and better still greater than or equal to 100, the enantioselectivity coefficient E being defined by the following formula:

$$E = \frac{\ln [(1-c)(1-ee_s)]}{\ln [(1-c)(1+ee_s)]}$$

with c: conversion rate

ee_s: enantiomeric excess of the residue substrate after enzymatic hydrolysis.

The present invention defines hereinafter, and implements in the examples, a "single-phase" process and a "two-phase" process, which correspond to two embodiments. The EH activity of the reference protein, like that of a "variant", "homolog" or "derivative", can be evaluated, for a given substrate, on the basis of these processes, and a comparison can be carried out between the performance levels of the reference protein and those of the other protein(s) tested.

An EH protein (reference protein, "variant", "homolog" or "derivative") can also be evaluated by reproducing the following test: 5.0 mg of epoxide substrate are dissolved in 3 ml of DMSO; 3 µl of 3-(trifluoromethyl)acetophenone (internal standard) and 7.9 ml of distilled water are added; an enzymatic solution is prepared: for example, 2 mg of enzymatic extract of the EH to be evaluated (purity of the order of 25%) are dissolved in 2.320 ml of distilled water; the solutions are placed at 27°C for 30 min; 100 µl of enzymatic solution are subsequently added to the reaction medium; samples taken regularly from the reaction medium make it possible to follow, over the course of the reaction, the formation of the diol: 400 µl of the reaction medium are added to 200 µl of acetonitrile; after vortexing, extraction is carried out with 400 µl of isooctane; for each sample, the organic phase is injected into chiral GC in order to measure the enantiomeric excess of the residue epoxide and the aqueous phase is injected onto reverse-phase HPLC so as to assay the formation of the diol (Nucleodur Chrompack Column; eluent: _CH_3CN/H_2O 60/40; flow rate

0.5 ml.min^{-1}; λ = 264 nm; injection 30 µl); by virtue of this method, the parameters ee and c are obtained, which makes it possible to calculate the value of E; the activity (diol formed) is also obtained. A comparison can be made between the performance levels of the reference protein and those of the other protein(s) tested.

According to an advantageous embodiment, such a test, or a similar test, e.g. developed from the present description, makes it possible to determine, in a panel of at least two proteins with EH activity, that (or those) which prove(s) to be the most effective on a given substrate. More broadly, such a test or a similar test makes it possible to determine the proteins, and the "variants", "homologs" and "derivatives", capable of producing, for a given substrate, a coefficient E greater than or equal to 10, preferably greater than or equal to 30. The present invention therefore allows those skilled in the art to select the enzyme most suitable for the substrate that they wish to convert. Such a test or a similar test also allows those skilled in the art to evaluate the impact of modifications introduced into the sequence of the enzyme, e.g. into the sequence SEQ ID NO: 2, and therefore in particular to be able to evaluate the modifications and mutations carried out for the purposes of improving the performance levels of the enzyme.

Entirely preferably, the protein has an EH activity on a CF_3 epoxide which is identical or substantially identical to the reference epoxide hydrolase.

As will be seen below, such a protein may be of natural origin, coming, for example, from an organism of the *Aspergillus* genus, from another microscopic fungus or from any other live source (bacterium, yeast, plant, etc.) or else of synthetic or recombinant origin. A protein of natural origin may have been modified so as

to give a synthetic or recombinant derived protein or polypeptide.

The "variant", "homologous" or "derived" proteins can be defined as comprising:

i. the sequences having a percentage homology of greater than equal to 40%, preferably greater than or equal to 80%, more preferably greater than or equal to 85%, even more preferably greater than or equal to 90%, and even better still greater than or equal to 95, 96, 97, 98 or 99%, with SEQ ID NO: 2, the protein thus defined having an EH activity on CF_3 epoxides;

ii. the sequences comprising at least 10, preferably at least 20, more preferably at least 50 or 100, consecutive amino acids of SEQ ID NO: 2 or of a sequence as defined in i, the protein thus defined having an EH activity on CF_3 epoxides.

According to a preferred embodiment, the invention uses such a "variant", "homologous" or "derived" protein which has, for a given substrate, an enantioselectivity coefficient E greater than or equal to 10, preferably greater than or equal to 30.

The term "homology" preferably refers to the identity between the amino acids compared.

This notion of homology can also take into account the "conservative" substitutions, which are substitutions of amino acids of the same class, such as substitutions of amino acids having uncharged side chains (such as asparagine, glutamine, serine, threonine and tyrosine), of amino acids having basic side chains (such as lysine, arginine and histidine), of amino acids having acidic side chains (such as aspartic acid and glutamic acid), or of amino acids having apolar side chains (such as glycine, alanine, valine, leucine, isoleucine,

proline, phenylalanine, methionine, tryptophan and cysteine), these substitutions with similar amino acids not significantly harming the biological activity of the reference protein, and preferably resulting in a protein conserving or increasing the biological activity of the reference protein.

More generally, the expression "*variant, homologous or derived amino acid sequence*" is therefore intended to mean any amino acid sequence which differs from the reference sequence by substitution, deletion and/or insertion of an amino acid or of several amino acids, this sequence constituting a protein or a polypeptide having an EH activity on CF_3 epoxides, the modifications not significantly harming the biological activity of the reference protein, and preferably conserving the biological activity of the reference protein or increasing the biological activity compared with the reference protein.

It can therefore be a protein or polypeptide comprising or essentially consisting of a fragment of SEQ ID NO: 2 or of a sequence as defined in i, for example a fragment formed from amino acids 1-339 or a homologous sequence.

The homology is generally determined using sequence analysis software (for example, Blast Software, National Center for Biotechnology Information, U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda, MD 20894, USA; or Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Amino acid sequences are aligned so as to obtain the maximum degree of homology (i.e. identity or similarity). To the end, it may be necessary to artificially introduce gaps into the sequence. Once the optimal alignment has been produced, the degree of homology is established by recording all

the positions for which the amino acids of the two compared sequences are identical or similar, relative to the total number of positions.

Preferably, the "variant", "homologous" or "derived" proteins or polypeptides have the same length or substantially the same length as the reference sequences.

Also considered to be homologs are the allelic variants that come from or are derived from strains of microorganisms, e.g. of *A. niger*, which have a biological activity similar to the reference protein.

In accordance with the invention, the proteins or polypeptides can, moreover, be chemically or enzymatically modified so as to improve their stability or their bioavailability.

Production of the epoxide hydrolase

According to a first embodiment, the protein is provided in the form of a concentrated and/or purified preparation obtained from a culture of a producer microorganism, especially microscopic fungus, e.g. *A. niger*, in particular LCP521. This is then referred to as a "natural" protein. Such a preparation can be obtained by means of a step consisting in extracting the enzyme from a cell culture, e.g. by mechanical lysis (by example, passage through a French press) or chemical lysis (including enzymatic lysis), followed by a step consisting in eliminating the cell debris and in recovering the liquid phase, which can comprise an appropriate centrifugation (preferably at low speed, e.g. of the order of 10 000 g) and/or filtration step, with recovery of the centrifugation supernatant or of the filtrate. It is preferred to subsequently concentrate the enzyme, e.g. by ultrafiltration. It is also preferred to carry out a purification of the

enzyme, and this can be performed by chromatography methods, in particular by successive passages over ion exchange and/or exclusion columns, for instance DEAE-sepharose, phenyl-sepharose, Mono Q and Superose 12, etc. Typically, the preparation is derived from an extraction, followed by centrifugation, recovery of the supernatant, and then concentration and preferably purification. The producer microorganism can be modified by genetic engineering so as to overexpress the enzyme, as described below.

According to a second embodiment, the protein is a "recombinant" protein, and this recombinant protein can optionally be in the form of a fusion protein. A recombinant protein can be produced by means of a process in which a vector containing a nucleic acid encoding the protein is transferred into a host cell, which is cultured under conditions that allow the expression of the corresponding protein. The recombinant protein produced is subsequently recovered and purified.

Such proteins can be produced in eukaryotic or prokaryotic systems according to the usual molecular biology, microbiology and recombinant DNA techniques, which are entirely known to those skilled in the art. These techniques are explained in detail in the literature. Reference may, for example, be made to: Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook et al., 1989"); DNA Cloning: A Practical Approach, Volumes I and II (D.N. Glover ed. 1985); Oligonucleotide Synthesis (M.J. Gait ed. 1984); Nucleic Acid Hybridization, A Practical Approach [B.D. Hames & S.J. Higgins eds. (1985) IRL Press, Oxford]; Transcription and Translation [B.D. Hames & S.J. Higgins eds. (1984) IRL Press, Oxford]; Animal Cell Culture [R.I. Freshney, ed. (1986)]; Immobilized Cells

and Enzymes [IRL Press, (1986)]; B. Perbal, A Practical Guide to Molecular Cloning (1984); F.M. Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (1994).

This embodiment provides for the expression of the protein, in the eukaryotic or prokaryotic host cells, from a nucleotide sequence encoding this protein. Such a nucleotide (or nucleic acid) sequence is in particular represented by:

(a) a nucleotide sequence comprising the nucleotide sequence represented in SEQ ID NO: 1 (which encodes the EH having the sequence SEQ ID NO: 2, i.e. encodes the reference protein);

(b) a nucleotide sequence which encodes the EH having the amino acid sequence SEQ ID NO: 2;

(c) a nucleotide sequence which differs from the sequence according to (a) or (b) by virtue of the degeneracy of the code;

or by a nucleotide sequence encoding a "variant", "homologous" or "derived" protein, in particular as defined above, and, for example:

(d) a nucleotide sequence which hybridizes to a sequence according to (a), (b) or (c), and encoding a protein having an EH activity on CF_3 epoxides;

(e) a nucleotide sequence having a percentage identity of greater than or equal to 45%, preferably greater than or equal to 80%, more preferably greater than or equal to 85%, even more preferably greater than or equal to 90%, and even better still greater than or equal to 95, 96, 97, 98 or 99%, with SEQ ID NO: 1, and encoding a protein having an EH activity on CF_3 epoxides;

(f) a fragment of a nucleotide sequence according to (a), (b), (c), (d) or (e), comprising at least 30, preferably at least 60, more preferably at least 150 or 300, consecutive nucleotides, and encoding a protein having an EH activity on CF_3 epoxides.

The proteins thus encoded have an EH activity on a CF_3 epoxide, and preferably a biological activity that is at least identical, similar or analogous to the reference protein on the same substrate. In the case of application to an enantioselective hydrolysis, the preferred proteins are those that have, for a given substrate, an enantioselectivity coefficient E greater than or equal to 10, preferably greater than or equal to 30.

According to characteristic d), the nucleotide sequence may be a nucleotide sequence which hybridizes to a sequence according to (a), (b) or (c), and encoding a protein having an EH activity on CF_3 epoxides; the hybridization conditions are preferably high stringency conditions, a term for which the definition is well known to those skilled in the art, who can refer to general manuals such as Sambrook et. al., 1989 and Hames & Higgins (1985) above.

As regards characteristic e), the identity between nucleotide sequences is generally determined using sequence analysis software (for example, Blast Software or Sequence Analysis Software Package, mentioned above) which takes into account the nucleotides that differ between two compared sequences and the nucleotides that are absent on one of the two sequences. The percentage value is given from the number of identical nucleotides over the total number of nucleotides of the reference sequence.

A homologous nucleotide sequence therefore includes any nucleotide sequence which differs from sequence SEQ ID NO: 1 by mutation, insertion, deletion or substitution of one or more bases, or by virtue of the degeneracy of the genetic code, provided that it encodes a peptide having the EH activity on CF_3 epoxides, the modifications not significantly harming the biological activity of the reference protein, and preferably conserving the biological activity of the reference protein or increasing the biological activity compared with the reference protein.

According to a preferred embodiment, use is made of the nucleotide sequence according to characteristic (a) or (b), even more preferentially according to characteristic (a). The sequence may also be a sequence comprising or essentially consisting of a fragment of such a sequence (a) or (b), for example nucleotides 1-1197.

As is entirely known in itself (see references above), the nucleotide sequence can be inserted into an expression vector, in which it is functionally linked to one or more element(s) allowing its expression or the regulation of its expression, such as, in particular, promoters, activators and/or transcription terminators.

The signals controlling the expression of the nucleotide sequences (promoters, activators, termination sequences, etc.) are selected according to the cellular host used. To this effect, the nucleotide sequences according to the invention can be inserted into vectors which replicate autonomously in the selected host, or vectors which integrate into the selected host. Such vectors will be prepared according to the methods commonly used by those skilled in the art, and the clones resulting therefrom can be introduced into an appropriate host by standard

methods, such as, for example electroporation or calcium phosphate precipitation.

The host cells can be transiently or stably transfected with these expression vectors. The cells can be obtained by introducing, into prokaryotic or eukaryotic host cells, a nucleotide sequence inserted into a vector as defined above, and then culturing said cells under conditions that allow the replication and/or the expression of the transfected nucleotide sequence.

Examples of such host cells include, in particular, mammalian cells, such as COS-7, 293 or MDCK cells, insect cells such as SF9 cells, bacteria such as E. coli, and yeast strains such as *Saccharomyces cerevisiae* or filamentous fungi such as *Aspergillus niger*.

The purification methods used are known to those skilled in the art. The recombinant protein obtained can be purified from cell lysates and extracts, from the culture medium supernatant, by methods used individually or in combination, such as fractionation, chromatography methods, immunoaffinity techniques using specific monoclonal or polyclonal antibodies, etc.

A recombinant epoxide hydrolase corresponding to the enzyme of *A. niger* LCP521 is commercially available under the reference "Epoxide Hydrolase, Aspergillus niger sp., recombinant from *Aspergillus niger*", BioChemika, catalog Fluka, code 71832.

The protein with EH activity can also be produced by chemical synthesis. To this effect, use may be made of any method well known to those skilled in the art. The peptide of the invention can, for example, be synthesized by synthetic chemistry techniques, such as Merrifield-type synthesis, which is advantageous for reasons of purity, antigenic specificity and absence of

unwanted byproducts, and because of its ease of production.

For its use in the conversion process of the invention, the protein can be in solution or immobilized on an appropriate solid support, such as, for example, DEAE cellulose or DEAE sepharose, Eupergit or modified Eupergit (C. Mateo et al., *Org. Biomol. Chem., 1,* 2739-2743 (2003)).

It is also possible to use, directly in the process, whole cells of the fungus such as *Aspergillus niger,* genetically modified so as to overexpress the epoxide hydrolase at a satisfactory level. This can be carried out by functionally inserting, into the genome of the fungus, one or more expression cassettes containing the coding sequence defined above, under the control of one or more element(s) allowing its expression or the regulation of its expression, such as, in particular, promoters, activators and/or transcription terminators. It is also possible to choose to modify the level of expression of the gene encoding the epoxide hydrolase, for example by insertion of a strong heterologous promoter and/or an activator so as to control the gene *in situ*. Finally, it is also possible to introduce into the fungus a nonintegrating expression vector, as described above. As described above, such a recombined microorganism can also be used for the production of enzyme *in vitro,* in which case the method for extracting the enzyme produced is subsequently carried out.

Conditions of the conversion process

The epoxide substrate to be converted is preferably dissolved in an appropriate solvent, preferably an appropriate organic solvent. The substrate can be a racemic epoxide or a nonracemic epoxide.

According to a first embodiment, the solvent for the substrate is a water-miscible organic solvent and the process is referred to as a "single-phase" process. This solvent is, by definition, capable of dissolving the epoxide substrate and is compatible with the enzyme (the solvent does not notably degrade the enzyme nor its epoxide hydrolase activity with respect to the CF_3 epoxide). The substrate can be used at any concentration, within the limits of its solubility in the solvent.

Examples of solvents for the single-phase system comprises DMSO (dimethyl sulfoxide), DMF (dimethyl-formamide), acetone, THF (tetrahydrofuran) dioxane and propanol. Preferred solvents are DMSO, DMF (dimethyl-formamide) and acetone. Two or more of these solvents can be used as a mixture.

The method subsequently comprises mixing the substrate solution and the enzyme, preferably the enzyme in a buffered solution, and preferably in water.

The isolated enzyme is preferably used in an aqueous solution, in particular water, e.g. distilled and/or purified water. As a result, when it is in solid form, e.g. pulverulent form, it is first dissolved in the aqueous solution.

The optimal concentrations of enzyme can be determined for each substrate and can vary within quite broad proportions. In general, the process can be carried out in a single-phase system with low concentrations of enzyme, for example of the order of from 1 to 60 mg of enzyme per liter of reaction medium, preferably from 5 to 30 mg/l.

For each substrate, it is possible to continuously control the duration of the hydrolysis reaction. For a new substrate, a preliminary experimental study makes

it possible to obtain the control parameters. The enantioselectivity of the hydrolysis reaction by the enzyme is due to a greater affinity and a greater catalytic constant for the (R) enantiomer compared with the (S) enantiomer, or according to the case, for the (S) enantiomer compared with the (R) enantiomer. The two enantiomers can be hydrolyzed, but, since the rate of hydrolysis of one of the enantiomers is considerably greater, controlling the duration of the reaction makes it possible to control the reaction. It is therefore quite obvious that those skilled in the art are capable of readily determining the optimal duration conditions for a given substrate. This can be done by taking regular samples of the reaction medium, on which the change in enantiomeric excess (ee) and the conversion rate (c) are evaluated. The examples give a procedure using acetonitrile to stop the reaction (see below) in each sample taken and isooctane extraction for passage onto gas chromatography (GC). While the duration of the hydrolysis phase can vary within broad proportions according in particular to the substrate considered and to the concentration of enzyme, it is nevertheless possible to specify, by way of indication, that the duration of the hydrolysis phase may generally be between 10 and 300 min, preferably between 25 and 80 minutes.

The conversion reaction can be stopped by any appropriate chemical or physical means, such as, for example: addition of a solvent that is toxic for the EH (acetonitrile, for example); addition of a base or of an acid, of detergent, of salt, etc.; or else by freezing, heating, microwave, microfiltration, etc.

According to a second embodiment, the solvent for the substrate is an organic solvent which is water-immiscible and the process is referred to as a "two-phase" process. This solvent is, by definition, capable of dissolving the epoxide substrate and is compatible

with the enzyme (the solvent does not notably degrade the enzyme or its epoxide hydrolase activity with respect to the CF_3 epoxide).

These water-immiscible solvents can be selected from alkanes, for example isooctane and hexane, cycloalkanes (cyclohexane, for example) and aromatic compounds (toluene, for example). Two or more of these solvents can be used as a mixture.

In this second embodiment, a particular mode consists in using in addition a water-miscible organic solvent, in particular a solvent as defined for the single-phase system.

In this second embodiment, an emulsion is preferably formed from the epoxide solution and the enzyme solution. The emulsion can be formed at the time the substrate solution (organic phase) is mixed with an aqueous solution of the enzyme (aqueous phase). It is also possible to form a preemulsion by mixing the substrate solution (organic phase) with water or an appropriate aqueous solution, this emulsion subsequently being mixed with the aqueous solution of the enzyme. The mixing means are such that an emulsion can form.

The epoxide substrates generally have solubility coefficients that are greater in the organic solvents used in this embodiment than in an aqueous solution containing water-miscible solvents of the single-phase mode. The concentration of epoxide can therefore be greater, and, for example, can be between 1 and 1000 g of epoxide per liter of reaction medium, preferably between 10 and 500 g/l.

The organic phase advantageously represents from 1 to 60%, preferably from 5 to 50%, of the total volume of the emulsion. Ratios of between 1 and 20%, preferably

of between 5 and 15%, are mainly used when the concentration of epoxide in the reaction medium is less than or equal to 100 g/l. Above this, ratios of between 20 and 60%, preferably of between 20 and 40%, are preferentially used.

The concentration of enzyme in the aqueous phase can vary within broad proportions. It is between 0.002 and 3 g of pure enzyme per liter, and preferably between 0.002 and 0.5 g/l.

In this two-phased embodiment, it is also possible to determine beforehand and/or to continuously control the duration of the hydrolysis reaction for a given substrate. As described above, this can be done by taking regular samples of the reaction medium, on which the change in enantiomeric excess and the conversion rate are evaluated. The examples give a procedure using ethyl acetate and a passage by GC, which can be applied to each sample taken in order to follow the evolution of the reaction. The duration of the hydrolysis phase can vary within broad proportions, according to the operating conditions, in particular to the amount of enzyme used, and to the substrate to be converted. It can, however, be specified that the duration of the hydrolysis phase may generally be between a few minutes, e.g. of the order of 15 to 30 minutes, and several days.

The conversion reaction can be stopped by any appropriate means, such as the addition of ethyl acetate, of ethyl ether, of dichloromethane, etc., or else by the means and techniques mentioned for the single-phase system.

In the process of the invention, and in particular the two embodiments which have just been described, the temperature during the hydrolysis phase is generally

maintained between 4 and 50°C, preferably between 25 and 30°C.

The hydrolysis phase is carried out in an appropriate reactor equipped with suitable stirring or mixing means. The mixing or stirring parameters are selected so as to optimize the hydrolysis phase. According to a specific modality, the stirring conditions, in particular the speed of rotation of the stirring means, are controlled continuously or at regular intervals. In the two-phase system, this control makes it possible in particular to ensure that the reaction medium is maintained in the form of an appropriate emulsion.

According to a preferred modality, the stirring is maintained throughout the hydrolysis phase.

The pH can be maintained between 6 and 9, preferably between 6.5 and 7.5

Coreactants can be used to increase the stability of the EH. By way of preference, mention may be made of reducing agents such as β-mercaptoethanol or cysteine.

At the end of the hydrolysis phase, the substrate (mixture of epoxide and of diol) can be extracted by conventional methods known to those skilled in the art, for instance direct extraction or continuous extraction, etc.

The residue epoxide and the diol can be separated, from the product of the hydrolysis, optionally from a extract thereof, by conventional methods known to those skilled in the art, for example by distillation, column chromatography, liquid/liquid extraction, etc. Operations of this type are described in detail, for example, in example number 23.

As mentioned above, the enantiomerically enriched diol can be cyclized to its epoxide. Any known method can be used for the cyclization of the (R) or (S) diol. By way of example, the cyclization can be carried out in two steps, by the addition of 1 equivalent of tosyl chloride (TsCl) in the presence of tetrahydrofuran (THF), and then of sodium hydride (NaH). The enantiomeric hydrolysis and cyclization operation can be reperformed one or more times, so as to increase the enantiomeric excess of (R) or (S) epoxide or of diol.

The present invention therefore makes it possible to prepare mixtures comprising large enantiomeric excesses of (S) epoxide and (R) diol [or, conversely, of (R) epoxide and (S) diol], preparations comprising a large enantiomeric excess of (S) epoxide [or, conversely, of (R) epoxide], or preparations comprising a large enantiomeric excess of (R) diol [or, conversely, of (S) diol]. These preparations may be enantiopure or essentially enantiopure, i.e. may exhibit an enantiomeric excess of (R) or (S) epoxide and/or of (R) or (S) diol that is greater than or equal to 95, 96, 97, 98 or 99%, or even equal to 100%. These preparations, which can be mixtures of epoxide and of diol, or which can be derived from a separation between epoxide and diol, and optionally a cyclization of the diol, are other subjects of the present invention.

A subject of the present invention is therefore also the use of a protein with EH activity on a CF_3 epoxide in accordance with the invention, for preparing such mixtures and preparations, from a racemic or nonracemic epoxide. According to a preferred embodiment, the epoxide hydrolase of *Aspergillus niger* LCP521 is used, for example an extraction protein, a recombinant protein or a protein produced by chemical synthesis. According to another embodiment, use is made of a protein of another origin, or a "variant", "homolog" or "derivative" of the epoxide hydrolase of *Aspergillus*

niger LCP521, which has an EH activity on a CF_3 epoxide, and preferably a biological activity that is identical, similar or analogous to the epoxide hydrolase of *Aspergillus niger* LCP521 on the same substrate, or even an EH activity greater than the latter. Preferably, this protein with EH activity has, for a given substrate, an enantioselectivity coefficient E greater than or equal to 10, preferably greater than or equal to 30.

According to another embodiment, the hydrolysis is nonenantioselective or not very enantioselective. The invention can then relate, inter alia, to a process for the nonenantioselective hydrolysis of a racemic or nonracemic mixture of epoxide to diol, in which process the reaction is carried out in a single-phase or two-phase system as described above. According to a specific modality, a racemic epoxide is converted into a racemic diol. This process can be carried out, notably, under experimental conditions that are particularly mild and economical, avoiding in particular the use of a more or less concentrated inorganic acidic or basic medium. The selection of a protein with EH activity can be readily made on the basis of the test described above, or of a similar test, the aim being, this time, to determine and select the proteins giving, for a given substrate, a coefficient E less than 10.

Some of the epoxides and diols according to the invention are intermediates that are useful as intermediates for the synthesis of pharmaceutically active compounds. Another subject of the invention is therefore the application of the process according to the invention, for preparing intermediates for the synthesis of pharmaceutically active compounds or for preparing such pharmaceutically active compounds, that are in the form of an epoxide and/or a diol, preferably in the form of an (R) or (S) epoxide, which is

preferably enantiopure or enantiomerically enriched (i.e. exhibiting an enantiomeric excess of greater than or equal to 95, 96, 97, 98 or 99%, or even equal to 100%), or in the form of an (R) or (S) diol, preferably enantiopure or enantiomerically enriched (i.e. exhibiting an enantiomeric excess of greater than or equal to 95, 96, 97, 98 or 99%, or even equal to 100%).

More generally, the invention applies to the production of products of industrial interest with biological activity, or of intermediates of such products, for example in the plant protection and agrochemical fields, e.g. insecticidal products, anti-parasitic products, etc.

As pharmaceutically active compounds that can be obtained from the epoxides and the diols according to the invention, mention may be made, for example, of:

- BRL-35113 (CAS 90730-95-3);
- Clofuperol Hydrochloride (CAS 17230-8764)
- S-15511 (AN-2000-47942; J. Pharmacol. Exp. Ther. 295, No. 2, 753-60, 2000);
- Aprepitant (CAS 170729-80-3);
- Flumaxedol Hydrochloride ($C_{11}H_{13}ClF_3NO$);
- Iralukast (CAS 15181-24-7; Novartis AG);
- Mabuterol Hydrochloride ($C_{13}H_{19}Cl_2F_3N_2O$);
- Oxaflozane Hydrochloride (CAS 26629-86-7);
- S-15261 (AN-94-44449; Diabetologia 37, No. 10, 969-75, 1994);
- Fludorex (CAS 15221-81-5);
- Flumetramide (CAS 7125-73-7);
- Fluminorex (CAS 720-76-3); and
- L-709210, L-732138, L-733060, L-636281, L-740141, L-741671, L-742311, L-742694, or L-758298 (Merck & Co Inc).

A subject of the present invention is also a composition that is useful for implementing the

conversion process according to the invention, comprising, for successive or simultaneous addition, a fluorinated epoxide in accordance with the invention, comprising one or more CF_3 groups, and a solvent for this epoxide, i.e. a water-miscible or water-immiscible organic solvent, as described above. According to a specific characteristic, this composition also comprises, for successive or simultaneous addition, an enzyme in accordance with the present invention.

A subject of the present invention is also a composition that is useful for implementing the conversion process according to the invention, comprising, for successive or simultaneous addition, an enzyme in accordance with the invention and a water-miscible or water-immiscible organic solvent as described above. According to a supplementary characteristic, this composition can comprise an aqueous solution.

The invention will now be described in greater detail by means of nonlimiting examples of application.

Examples

In the examples which follow, the enzyme used is the epoxide hydrolase of *A. niger* (An EH) strain LCP521. This enzyme is a recombinant protein produced in a strain of *Aspergillus niger* in accordance with the process described in part B) "Cloning and characterization of the soluble epoxide hydrolase of *Aspergillus niger* which is related to mammalian microsomal epoxide hydrolases" in WO-A-00 68394. An enzyme thus produced is commercially available as mentioned above.

Kinetic resolution by An EH - single-phase system

Example 1

Kinetic resolution of 4-(trifluoromethoxyphenyl) oxirane by An EH - single-phase system

5.1 mg of 4-(trifluoromethoxyphenyl) oxirane are dissolved in 3 ml of DMSO. 3 µl of 3-(trifluoromethyl)acetophenone (internal standard) and 7.9 ml of distilled water are added ([4-(trifluoromethoxyphenyl) oxirane] = 2.5 mM; %DMSO = 30). An enzymatic solution is prepared: 2 mg of enzymatic extract of recombinant *An* EH (characterized as having a purity of the order of 25%) are dissolved in 4.550 ml of distilled water. The solutions are placed at 27°C for 30 min. 100 µl of enzymatic solution are subsequently added to the reaction medium.

Samples of the reaction medium taken regularly make it possible to monitor, over the course of the reaction, the enantiomeric excess of the residue substrate and its rate of conversion (400 µl of the reaction medium are added to 200 µl of acetonitrile. After vortexing, extraction is carried out with 400 µl of isooctane and 2 µl of the organic phase are injected onto chiral GC). The values obtained correspond to a value of the apparent enantioselectivity coefficient E of 30.

The enantioselectivity coefficient E is defined as being:

$$E = \frac{\ln [(1-c)(1-ee_s)]}{\ln [(1-c)(1+ee_s)]}$$

with c: conversion rate

ee_s: enantiomeric excess of the residue substrate after enzymatic hydrolysis.

Analyses carried out on a column of Chirasil-Dex CB type (T = 120°C; tr = 4.4 min and 4.8 min for the two enantiomers of the epoxide; tr = 3.9 min for the internal standard).

Example 2

The procedure described in example 1 is applied to 2-(trifluoromethylphenyl) oxirane (4.7 mg, i.e. [2-(trifluoromethylphenyl) oxirane] = 2.5 mM), in the presence of 25% of DMSO. The enzymatic solution added (100 µl) contains 2 mg of enzymatic extract of the recombinant *An* EH (characterized as having a purity of the order of 25%) in 2.470 ml of distilled water.
The values obtained correspond to an apparent enantioselectivity coefficient E of 5.

Analyses carried out on a column of Lipodex E type (T = 100°C; tr = 3.8 min and 4.8 min for the two enantiomers of the epoxide; tr = 4.3 for the internal standard).

Example 3

The procedure described in example 1 is applied to 3-(trifluoromethylphenyl) oxirane (5.0 mg, i.e. [3-(trifluoromethylphenyl) oxirane] = 2.66 mM), in the presence of 20% of DMSO. The enzymatic solution added (100 µl) contains 2 mg of enzymatic extract of the recombinant *An* EH (characterized as having a purity of the order of 25%) in 2.680 ml of distilled water.
The values obtained correspond to an enantioselectivity coefficient E of 10.

Analyses carried out on a column of Chirasil-Dex CB type (T = 90°C; tr = 13.2 min and 13.7 min for the two enantiomers of the epoxide; tr = 10.4 min for the internal standard).

Example 4

The procedure described in example 1 is applied to 4-(trifluoromethylphenyl) oxirane (4.7 mg, i.e.

[4-(trifluoromethylphenyl) oxirane] = 2.5 mM), in the presence of 20% of DMSO. The enzymatic solution added (100 µl) contains 2 mg of enzymatic extract of the recombinant *An* EH (characterized as having a purity of the order of 25%) in 2.470 ml of distilled water.
The values obtained correspond to an apparent enantioselectivity coefficient E of 50.

Analyses carried out on a column of Chirasil-Dex CB type (T = 120°C; tr = 9.7 min and 10.7 min for the two enantiomers of the epoxide; tr = 3.9 min for the internal standard).

Example 5

The procedure described in example 1 is applied to 3,5-(bistrifluoromethylphenyl) oxirane (0.77 mg, i.e. [3,5-(bistrifluoromethylphenyl) oxirane] = 0.3 mM), in the presence of 25% of DMSO. The enzymatic solution added (100 µl) contains 2 mg of enzymatic extract of the recombinant *An* EH (characterized as having a purity of the order of 25%) in 15.065 ml of distilled water.

The values obtained correspond to an apparent enantioselectivity coefficient E of 4.

Analyses carried out on a column of Chirasil-Dex CB type (T = 80°C; tr = 9.8 min and 10.1 min for the two enantiomers of the epoxide; tr = 17.1 min for the internal standard).

Example 6

The procedure described in example 1 is applied to methyl-(3-trifluoromethylphenyl) oxirane (5.0 mg, i.e. [methyl-3-trifluoromethylphenyl) oxirane] = 2.5 mM), in the presence of 30% of DMSO. The enzymatic solution added (100 µl) contains 2 mg of enzymatic extract of

the recombinant *An* EH (characterized as having a purity of the order of 25%) in 2.320 ml of distilled water. The values obtained correspond to an enantioselectivity coefficient of 25.

Analyses carried out on a column of Chirasil-Dex CB type (T = 100°C; tr = 8.5 min and 9.1 min for the two enantiomers of the epoxide; tr = 6.9 min for the internal standard).

Example 7

The procedure described in example 1 is applied to methyl-(4-trifluoromethylphenyl) oxirane (3.6 mg, i.e. [methyl-(4-trifluoromethylphenyl) oxirane] = 1.8 mM), in the presence of 30% of DMSO. The enzymatic solution added (100 µl) contains 2 mg of enzymatic extract of the recombinant *An* EH (characterized as having a purity of the order of 25%) in 3.222 ml of distilled water. The values obtained correspond to an apparent enantioselectivity coefficient E of 30.

Analyses carried out on a column of Chirasil-Dex CB type (T = 120°C; tr = 6.4 min and 7.1 min for the two enantiomers of the epoxide; tr = 3.9 min for the internal standard).

Example 8

The procedure described in example 1 is applied to 4-(trifluoromethylthiophenyl) oxirane (4.4 mg, i.e. [4-(trifluoromethylthiophenyl) oxirane] = 2 mM), in the presence of 30% of DMSO. The enzymatic solution added (100 µl) contains 2 mg of enzymatic extract of the recombinant *An* EH (characterized as having a purity of the order of 25%) in 5.273 ml of distilled water. The values obtained correspond to an apparent enantioselectivity coefficient E of 160.

Analyses carried out on a column of Chirasil-Dex CB type (T = 130°C; tr = 7.2 min and 7.6 min for the two enantiomers of the epoxide; tr = 2.8 min for the internal standard).

Kinetic resolution by An EH - two-phase system - analytical scale

Example 9

Kinetic resolution of 4-(trifluoromethoxyphenyl) oxirane by An EH - two-phase system; analytical scale

100 mg of *4-(trifluoromethoxyphenyl) oxirane* are placed in 100 µl of isooctane in a flat-bottomed tall narrow tube (15 mm diameter). 20 µl of 3-(trifluoromethyl)acetophenone (internal standard) and 850 µl of distilled water are added. The flask is stoppered, mixed very vigorously by magnetic agitation (formation of an emulsion) and placed at 27°C. Before the addition of 0.86 mg of enzymatic extract of the recombinant An EH (purity 25%) in solution in 50 µl of distilled water, the agitation is stopped, 1 µl of the organic phase is removed, and the agitation is reinitiated. The sample is diluted with ethyl acetate and injected into chiral GC. This type of sample, taken repeatedly over time, makes it possible to monitor the change in enantiomeric excess of the residue substrate and in the epoxide conversion rate. The values obtained correspond to an apparent enantioselectivity coefficient E of 160.

Analyses carried out on a column of Chirasil-Dex CB type (T = 120°C; tr = 4.4 min and 4.8 min for the two enantiomers of the epoxide; tr = 3.9 min for the internal standard).

Example 10

The procedure described in example 9 is applied to 2-(trifluoromethylphenyl) oxirane. In this case, 1.7 mg of enzymatic extract of the recombinant An EH (purity 25%) are added per 100 mg of substrate.
The values obtained correspond to an apparent enantioselectivity coefficient E of 20.

Example 11

The procedure described in example 9 is applied to 3-(trifluoromethylphenyl) oxirane. In this case, 1.7 mg of enzymatic extract of the recombinant An EH (purity 25%) are added per 100 mg of substrate.
The values obtained correspond to an apparent enantioselectivity coefficient E of 10.

Example 12

The procedure described in example 9 is applied to 4-(trifluoromethylphenyl) oxirane. In this case, 1.7 mg of enzymatic extract of the recombinant An EH (purity 25%) are added per 100 mg of substrate.
The values obtained correspond to an apparent enantioselectivity coefficient E of 270.

Example 13

The procedure described in example 9 is applied to (3,5-bistrifluoromethylphenyl) oxirane. In this case, 1.7 mg of enzymatic extract of the recombinant An EH (purity 25%) are added per 100 mg of substrate.
The values obtained correspond to an apparent enantioselectivity coefficient E of 17.

Example 14

The procedure described in example 9 is applied to methyl-(3-trifluoromethylphenyl) oxirane. In this case,

1.7 mg of enzymatic extract of the recombinant An EH (purity 25%) are added per 100 mg of substrate.
The values obtained correspond to an apparent enantioselectivity coefficient E of 25.

Example 15

The procedure described in example 9 is applied to methyl-(4-trifluoromethylphenyl) oxirane. In this case, 1.7 mg of enzymatic extract of the recombinant An EH (purity 25%) are added per 100 mg of substrate.
The values obtained correspond to an apparent enantioselectivity coefficient E of 50.

Kinetic resolution via a chemical method (comparative)

Example 16

Kinetic resolution of 4-(trifluoromethoxyphenyl) oxirane via a chemical method

204 mg (1 mmol) of 4-(trifluoromethoxyphenyl) oxirane are added to 4.76 mg (0.007 mmol) of (*R,R*)(Salen)Co(OAa), in a 1 ml minireactor equipped with magnetic stirring. 9.9 µl of water (0.55 mmol) are introduced at ambient temperature in a single step. The system is placed at ambient temperature for 48 h, with stirring. The entire reaction medium is subsequently extracted with 10 ml of isooctane. 1 ml of the organic phase is then removed. 1 ml of isooctane solution containing the 3-(trifluoromethyl)acetophenone standard at 5 $g.l^{-1}$ is then added thereto. 0.2 µl of this mixture is injected into chiral GC.

The values obtained ($ee_{residue\ epoxide}$ = 98.6%; $ee_{diol\ formed}$ = 89.0%) correspond to an apparent enantioselectivity coefficient E of 84.

Example 17

The procedure described in example 16 is applied to (2-trifluoromethylphenyl) oxirane on a millimole scale.
The values obtained ($ee_{residue\ epoxide}$ = 23.3%; c = 77.7%) correspond to an apparent enantioselectivity coefficient E of 1.4.

Example 18

The procedure described in example 16 is applied to (3-trifluoromethylphenyl) oxirane on a millimole scale.
The values obtained ($ee_{residue\ epoxide}$ = 100%; c = 61%) correspond to an apparent enantioselectivity coefficient E of greater than or equal to 33.

Example 19

The procedure described in example 16 is applied to (4-trifluoromethylphenyl) oxirane on a millimole scale.
The values obtained ($ee_{residue\ epoxide}$ = 97.8%; $ee_{diol\ formed}$ = 79.3%) correspond to an apparent enantioselectivity coefficient E of 38.

Example 20

The procedure described in example 16 is applied to methyl-(3-trifluoromethylphenyl) oxirane on a millimole scale.
The values obtained ($ee_{residue\ epoxide}$ = 0,1%; c = 2.0%) correspond to an apparent enantioselectivity coefficient E of 1.

Example 21

The procedure described in example 16 is applied to methyl-(4-trifluoromethylphenyl) oxirane on a millimole scale.
The values obtained ($ee_{residue\ epoxide}$ = 0.2%; c = 19.4%) correspond to an apparent enantioselectivity coefficient E close to 1.

Example 22

The procedure described in example 16 is applied to 4-(trifluoromethylthiophenyl) oxirane on a millimole scale.

The values obtained ($ee_{residue\ epoxide}$ = 99.6%; $ee_{diol\ formed}$ = 89.4%) correspond to an apparent enantioselectivity coefficient E of 110.

Kinetic resolution by An EH - two-phase system - preparative scale

Example 23

Kinetic resolution of 4-(trifluoromethoxyphenyl) oxirane by An EH - two-phase system; preparative scale

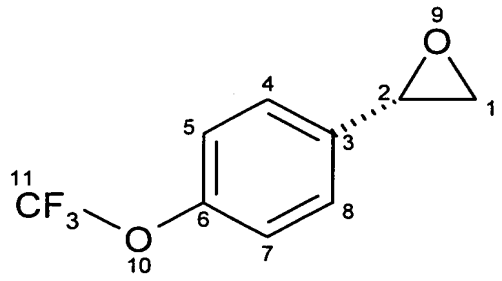
1.25 g of racemic 4-(trifluoromethoxyphenyl) oxirane (i.e. 6.13 mmol) in 2.5 ml of isooctane are placed in a flask equipped with mechanical stirring. 21.5 ml of distilled water are added. The entire mixture is placed in a bath thermostated at 27°C, with stirring. During this time, an enzymatic solution containing 12.5 mg per ml of water of recombinant *An* EH enzymatic extract (purity 25%) is prepared. This solution is itself also placed at 27°C. After 30 minutes, 1 ml of the enzymatic solution previously prepared is added to the reaction medium. This moment corresponds to time t_0.

The reaction is monitored by chiral GC. Samples of 1 µl are regularly taken from the reaction median, and are added to 40 µl of ethyl acetate placed beforehand in an eppendorf. After vortexing and centrifugation, the mixture is injected into chiral GC in order to measure the enantiomeric excess of the residue epoxide. When it reaches a sufficient value (enantiomeric excess > 97%), the reaction is stopped with adding 30 ml of ethyl acetate. The mixture is allowed to separate by settling out, the organic phase is recovered and the aqueous

phase is extracted with 2 × 50 ml of ethyl acetate. The organic phases are combined, washed with 30 ml of saturated aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The residue epoxide and the diol formed are separated by flash chromatography on silica gel (50 parts; eluent: 90/10 hexane/ethyl acetate up to pure ethyl acetate). With a view to measuring the optical rotations, each isolated product is subsequently subjected to bulb-to-bulb purification in order to remove all traces of solvent and of silica. 615 mg of (*R*)-(4-trifluoromethoxyphenyl)ethane-1,2-diol (yield = 45.6%; ee = 94.5%) and 543 mg of (*S*)-4-(trifluoromethoxyphenyl) oxirane (yield = 43.4%; ee = 98.6%) are obtained.

Structural analysis

(S)-4-(trifluoromethoxyphenyl) oxirane



^{1}H NMR/$CDCl_3$: δ 2.79 (dd. 1H, $^3J_{HH}$ = 2.5 Hz, $^1J_{HH}$ = 5.3 Hz, H_1), δ 3.19 (dd, 1H, $^3J_{HH}$ = 4.0 Hz, $^1J_{HH}$ = 5.3 Hz, H_1), δ 3.92 (dd, 1H, $^3J_{HH}$ = 2.5 Hz, $^3J_{HH}$ = 4.0 Hz. H_2), δ 7.38-7.65 (m, 4H, $H_{4,6,7,8}$).

^{13}C NMR/$CDCl_3$: δ 51.3 (C_1), δ 51.7 (C_2), δ 120.4 (q, $^1J_{CF}$ = 265.5 Hz, C_{11}), δ 121.1 (C_4 and C_8), δ 126.9 (C_5 and C_7), δ 136.4 (C_3), δ 149.1 (q, J = 1.9 Hz, C_6).

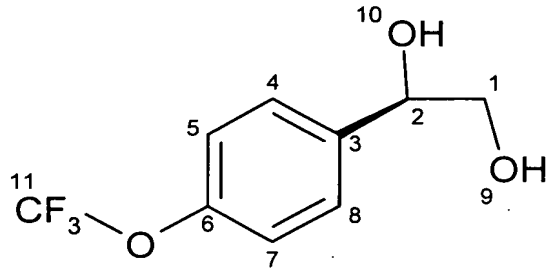
^{19}F {^{1}H} NMR/$CDCl_3$: δ -57.35.

HRMS: calculated for $C_9H_6F_3O_2$ (ES^- MS): 203.0320. Found: 203.0311.

Elemental analysis: Calculated: C: 52.95%; H: 3.46%; F: 27.92%. Found: C: 52.60%; H: 3.38%; F: 29.04%.

$[\alpha]_D^{22}$ = +13.7 (c 1.58; $CHCl_3$) [(*S*)/ee = 98.6%]

(*R*)-(4-trifluoromethoxyphenyl)ethane-1,2-diol



Mp: 64°C

^{1}H NMR/$CDCl_3$: δ 2.13 (unresolved peak, 1H, H_{10} or H_9) δ 2.69 (unresolved peak, 1H, H_{10} or H_9), δ 3.70 (m, 2H, $H_{1,1'}$), δ 4.84 (m, 1H, H_2), δ 7.21 (d, 2H, $^3J_{HH}$ = 8.75 Hz, $H_{4,8}$), δ 7.41 (d, 2H, $^3J_{HH}$ = 8.5 Hz, $H_{5,7}$).

^{13}C NMR/Acetone-d_6: δ 68.7 (C_1), δ 74.5 (C_2), δ 121.4 (C_5 and C_7), δ 121.5 (q, $^1J_{CF}$ = 253.3 Hz, C_{11}), δ 128.8 (C_4 and C_8), δ 143.1 (C_3), δ 148.9 (m, C_6)

^{19}F {^{1}H} NMR/$CDCl_3$: δ -57.61.

HRMS: calculated for $C_9H_8F_3O_3$ (ES^- MS): 221.0426. Found: 221.0437.

Elemental analysis: Calculated: C: 48.66%; H: 4.08%; F: 25.65%. Found: C: 48.79%; H: 4.08%; F: 26.41%.

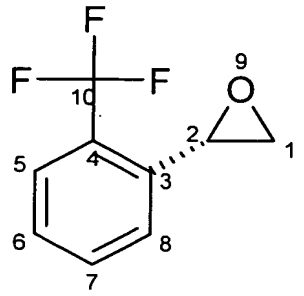
$[\alpha]_D^{22}$ = -41.5 (c 1.04; $CHCl_3$) [(*S*)/ee = 94.5%]

Example 24

The procedure described in example 23 is applied to 2-(trifluoromethylphenyl) oxirane. (*S*)-2-(trifluoromethylphenyl) oxirane (yield = 34.5%; ee = 97.9%) and (*R*)-2-trifluoromethylphenyl)ethane-1,2-diol (yield 59.2%; ee = 77.3%) are obtained.

Structural analysis

(*S*)-2-(trifluoromethylphenyl) oxirane



^{1}H NMR/DMSO-d_6: δ 2.75 (dd, 1H, $^1J_{HH}$ = 5.5 Hz, $^3J_{HH}$ = 2.5 Hz, H_1), δ 3.21 (dd, 1H, $^1J_{HH}$ = 5.5 Hz, $^3J_{HH}$ = 4.25 Hz $H_{1'}$), δ 4.15 (m, 1H, H_2), δ 7.44 (d, 1H, $^3J_{HH}$ = 7.75 Hz, H_8), δ 7.54 (t, $^3J_{HH}$ = 7.5 Hz, H_7), δ 7.63 (t, 1H, $^3J_{HH}$ = 7.5 Hz, H_6), δ 7.75 (d, 1H, $^3J_{HH}$ = 7.75 Hz, H_5).

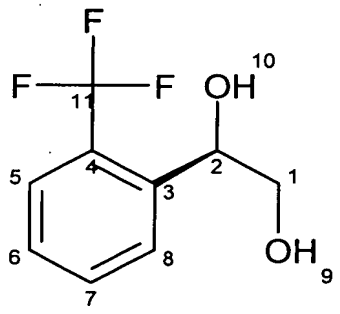
^{13}C NMR/DMSO-d_6: δ 48.5 (q, $^4J_{CF}$ = 2.7 Hz, C_2), δ 50.2 (C_1), δ 124.3 (q, $^1J_{CF}$ = 273.5, C_{10}), δ 125.5 (q, $^3J_{CF}$ = 5.4 Hz, C_5), δ 125.5 (m, C_6 or C_8), δ 126.9 (q, $^2J_{CF}$ = 30.7 Hz, C_4), δ 128.2 (C_7), δ 32.9 (m, C_6 or C_8), δ 136.2 (m, C_3).

^{19}F {^{1}H} NMR/$CDCl_3$: δ -59.62.

HRMS: calculated for $C_9H_6F_3O$ (ES^- MS): 187.0371. Found: 187.0374.

$[\alpha]_D^{22}$ = +62.4 (c 0.98; $CHCl_3$) [(*S*)/ee = 97.9%]

(*R*)-2-(trifluoromethylphenyl)ethane-1,2-diol



Mp: 51°C

^{1}H NMR/$CDCl_3$: δ 2.45 (s, 1H, H_9 or H_{10}), δ 3.00 (s, 1H, H_9 or H_{10}), δ 3.67 (unresolved peak, 2H, $H_{1,1'}$), δ 5.23 (unresolved peak, 1H, H_2), δ 7.44-7.65 (m, 4H, $H_{4,6,7,8}$).

^{13}C NMR/$CDCL_3$: δ 67.8 (C_1), δ 74.4 (C_2), δ 125.6 (q, $^3J_{CF}$ = 5.8 Hz, C_5), δ 124.2 (q, $^1J_{CF}$ = 273.7 Hz, C_{11}), δ 127.3 (q,

$^2J_{CF}$ = 30.3 Hz, C_4), δ 128.0 (C_7), δ 128.2 (m, C_6 or C_8), δ 132.2 (m, C_6 or C_8), δ 139.2 (C_3).

^{19}F {^1H} NMR/$CDCl_3$: δ -57.93.

HRMS: calculated for $C_9H_8F_3O_2$ (ES^- MS): 205.0476. Found: 205.0468.

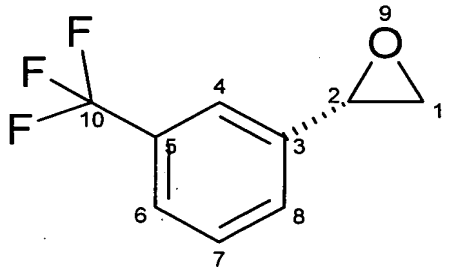
$[\alpha]_D^{22}$ = -47.9 (c 0.98; $CHCl_3$) [(*S*)/ee = 77.3%]

Example 25

The procedure described in example 23 is applied to 3-(trifluoromethylphenyl) oxirane. (*S*)-3-(trifluoromethylphenyl) oxirane (yield = 11.4%; ee = 98.7%) and (*R*)-3-(trifluoromethylphenyl)ethane-1,2-diol (yield = 84.9%; ee = 13.2%) are obtained.

Structural analysis

(*S*)-3-(trifluoromethylphenyl) oxirane



^1H NMR/$CDCl_3$: δ 2.79 (dd, 1H, $^3J_{HH}$ = 2.5 Hz, $^1J_{HH}$ = 5.3 Hz, H_1), δ 3.19 (dd, 1H, $^3J_{HH}$ = 4.0 Hz, $^1J_{HH}$ = 5.3 Hz, H_1), δ 3.92 (dd, 1H, $^3J_{HH}$ = 2.5 Hz, $^3J_{HH}$ = 4.0 Hz, H_2), δ 7.38-7.65 (m, 4H, $H_{4,6,7,8}$).

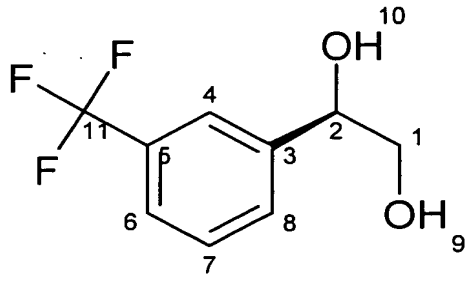
^{13}C NMR/$CDCl_3$: δ 51.4 (C_1), δ 51.7 (C_2), δ 122.3 (q, $^3J_{CF}$ = 3.8 Hz, C_4), δ 124.0 (q, $^1J_{CF}$ = 272.0 Hz, C_{10}), δ 125.0 (q, $^3J_{CF}$ = 3.7 Hz, C_6), δ 128.7 (m, C_7), δ 129.8 (C_8), δ 131.0 (q, $^2J_{CF}$ = 32.4 Hz, C_5), δ 138.8 (C_3).

^{19}F {^1H} NMR/$CDCl_3$: δ -62.34.

HRMS: calculated for $C_9H_6F_3O$ (ES^- MS): 187.1412. Found: 187.1324.

$[\alpha]_D^{22}$ = +9.1 (c 0.92; $CHCl_3$) [(*S*)/ee = 98.7%]. Configuration (*S*) according to M.J. Ferris, Arylethanolamine derivatives and their use in pharmaceutical compositions, EP 40,000, **1981**.

(*R*)-3-(trifluoromethylphenyl)ethane-1,2-diol



^{1}H NMR/$CDCl_3$: δ 2.26 (unresolved peak, 1H, H_9 or H_{10}), δ 2.87 (unresolved peak, 1H, H_9 or H_{10}), δ 3.57 (dd, 1H, ^1J = 11 Hz, ^3J = 8.25 Hz, H_1), δ 3.73 (dd, 1H, ^1J = 11 Hz, ^3J = 3.25 Hz, $H_{1'}$), δ 4.81 (dd, 1H, ^3J = 3.25 Hz, ^3J = 8.25 Hz, H_2) δ 7.44-7.65 (m, 4H, H aromatic).

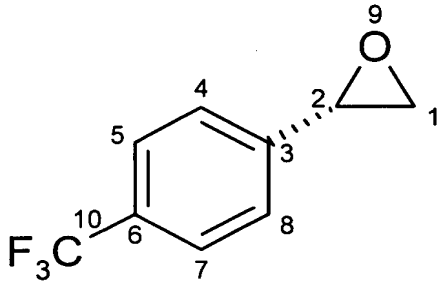
^{13}C NMR/$CDCl_3$: δ 67.9 (C_1), δ 74.0 (C_2), δ 122.8 (q, $^3J_{CF}$ = 3.8 Hz, C_4 or C_6), δ 124.8 (q, $^3J_{CF}$ = 3.7 Hz, C_4 or C_6), δ 124.0 (q, $^1J_{CF}$ = 272.4 Hz, C_{11}), δ 129.0 (C_7 or C_8), δ 129.4 (C_7 or C_8), δ 130.9 (q, $^2J_{CF}$ = 32.3 Hz, C_5), δ 139.4(C_3).
$[\alpha]_D^{22}$ = -5.7 (c 0.98; $CHCl_3$) [(*S*)/ee = 13.2%].

Example 26

The procedure described in example 23 is applied to 4-(trifluoromethylphenyl) oxirane. (*S*)-4-(trifluoromethylphenyl) oxirane (yield = 37.4%; ee = 97.9%) and (*R*)-4-(trifluoromethylphenyl)ethane-1,2-diol (yield = 51.3 % ; ee = 84.3%) are obtained.

Structural analysis

(*S*)-4-(trifluoromethylphenyl) oxirane



^{1}H NMR/$CDCl_3$: δ 2.77 (dd, 1H, $^1J_{HH}$ = 5.75 Hz, $^3J_{HH}$ = 2.75 Hz, H_1) δ 3.18 (dd, 1H, $^1J_{HH}$ = 5.75 Hz, $^3J_{HH}$ = 4.0 Hz, $H_{1'}$), δ 3.91 (dd, 1H, $^3J_{HH}$ = 4 Hz, $^3J_{HH}$ = 2.75 Hz, H_2) δ 7.39 (d, 1H, $^3J_{HH}$ = 8 Hz, H_4, H_8), δ 7.60 (d, 2H, $^3J_{HH}$ = 8 Hz, H_5, H_7).

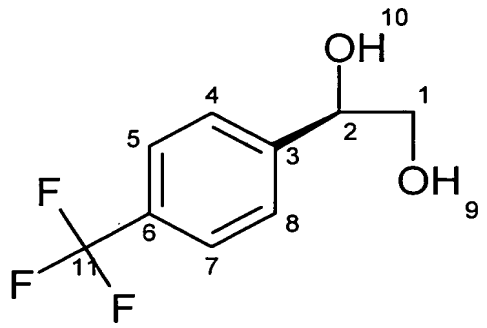
^{13}C NMR/$CDCl_3$: δ 51.4 (C_1), δ 51.7 (C_2), δ 124.0 (q, $^1J_{CF}$ = 271.8 Hz, C_{10}), δ 125.5 (q, $^3J_{CF}$ = 4.0 Hz, C_5 and C_7), δ 125.7 (C_4 and C_8), δ 130.4 (q, $^2J_{CF}$ = 32.2 Hz, C_6), δ 141 (s, C_3).

^{19}F {^{1}H} NMR/$CDCl_3$: δ -62.28.

HRMS: calculated for $C_9H_6F_3O$ (ES^- MS): 187.0371. Found: 187.0374.

[α]$_D^{22}$ = +18.0 (c 1.13; $CHCl_3$) [(*S*)/ee = 97.9%]

(*R*)-4-(trifluoromethylphenyl)ethane-1,2-diol



Mp: 101°C (Lit.: 93°C according to Hirose, K.; Ogasahara, K.; Nishioka, K.; Tobe, Y.; Naemura, K.; *J. Chem. Soc., Perkin Trans. 2* **2000**, 1984-1993. "Enantioselective complexation of phenoic crown ethers with chiral aminoethanol derivatives: effects of

substituents of aromatic rings of hosts and guests on complexation".

^{1}H NMR/$CDCl_3$: δ 2.07 (unresolved peak, 1H, H_{10} or H_9), δ 2.70 (unresolved peak, 1H, H_{10} or H_9), δ 3.88 (m, 2H, $H_{1,1'}$), δ 4.89 (m, 1H, H_2), δ 7.50 (d, 2H, $^3J_{HH}$ = 8.5 Hz, $H_{4,8}$), δ 7.63 (d, 2H, $^3J_{HH}$ = 8.5 Hz, $H_{5,7}$).

^{13}C NMR/Acetone-d_6: δ 68.6 (C_1), δ 74.7 (C_2), δ 125.5 (q, $^1J_{CF}$ = 269.4 Hz, C_{11}), δ 125.6 (q, $^3J_{CF}$ = 3.8 Hz, C_5 and C_7), δ 127.8 (C_4 and C_8), δ 129.5 (q, $^2J_{CF}$ = 31.9 Hz, C_6), δ 148.5 (C_3).

^{19}F {^{1}H} NMR/Acetone-d_6: δ -62.21.

HRMS: calculated for $C_9H_9F_3O_2$ (ES^- MS): 205.0476. Found: 205.0457.

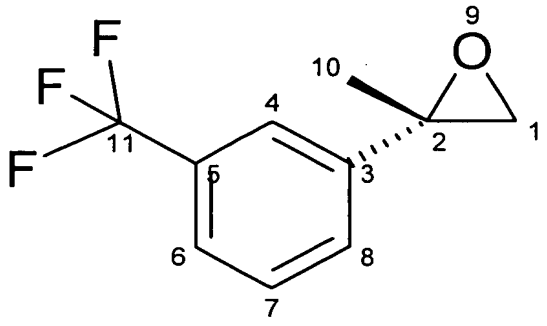
$[\alpha]_D^{22}$ = -39.3 (c 1.03; $CHCl_3$) [(*S*)/ee = 84.3%]. Configuration (*R*) according to Shimada, T.; Mukaide, K.; Shinohara, A.; Han, J.W.; Hayashi, T., Asymmetric Synthesis of 1-Aryl-1,2-ethanediols from Arylcetylenes by Palladium-catalyzed Asymmetric Hydrosilylation as a Key Step, *J. Am. Chem. Soc.*, **2002**, *124*, 1584-1585.

Example 27

The procedure described in example 23 is applied to methyl-(3-trifluoromethylphenyl) oxirane. (*S*)-methyl-(3-trifluoromethylphenyl) oxirane (yield = 32.7%; ee = 98.3%) and (*R*)-methyl-(3-trifluoromethylphenyl)ethane-1,2-diol (yield = 64.1%; ee = 59.0%) are obtained.

Structural analysis

(*S*)-methyl-(3-trifluoromethylphenyl) oxirane



^{1}H NMR/$CDCl_3$: δ 1.55 (s, 3H, H_{10}), δ 2.75 (d, 1H, $^1J_{HH}$ = 5.4 Hz, H_1), δ 2.98 (d, 1H, $^1J_{HH}$ = 5.4 Hz, H_1), δ 7.40-7.59 (m, 4H, $H_{4,6,7,8}$).

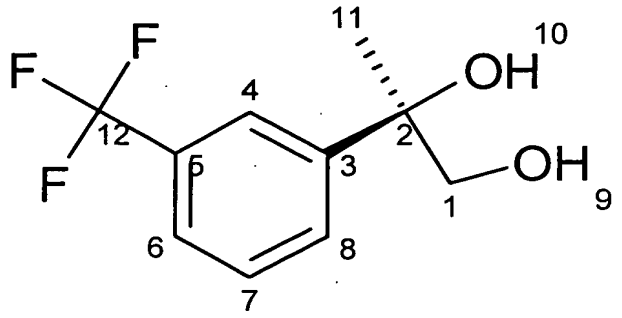
^{13}C NMR/$CDCl_3$: δ 21.5 (C_{10}), δ 56.2 (C_1), δ 57.0 (C_2), δ 122.2 (q, $^3J_{CF}$ = 3.8 Hz, C_4 or C_6), δ 124.1 (q, $^1J_{CF}$ = 272.5 Hz, C_{11}), δ 124.3 (q, $^1J_{CF}$ = 3.8 Hz, C_4 or C_6), δ 128.7 (C_7), δ 128.9 (C_8), δ 130.8 (q, $^2J_{CF}$ = 32.1 Hz, C_5), δ 142.4 (C_3).

^{19}F {^{1}H} NMR/$CDCl_3$: δ -62.27.

HRMS: calculated for $C_{10}H_8F_3O$ (ES^- MS): 201.0527. Found: 201.0533.

[α]$_D^{22}$ = +8.3 (c 1.0; $CHCl_3$) [(*S*)/ee = 98.3%].

(*R*)-methyl-(3-trifluoromethylphenyl)ethane-1,2-diol



Mp: 48°C.

^{1}H NMR/$CDCl_3$: δ 1.52 (s, 3H, H_{11}), δ 2.37 (unresolved peak, 1H, H_9 or H_{10}), δ 2.99 (unresolved peak, 1H, H_9 or H_{10}), δ 3.69 (m, 2H, $H_{1,1'}$), δ 7.43-7.73 (m, 4H, $H_{4,6,7,8}$).

^{13}C NMR/$CDCl_3$: δ 26.0 (C_{11}), δ 70.7 (C_1), δ 74.7 (C_2), δ 122.1 (q, $^3J_{CF}$ = 3.8 Hz, C_4 or C_6), δ 124.0 (q, $^1J_{CF}$ = 3.8 Hz, C_4 or

C_6), δ 124.2 (q, $^1J_{CF}$ = 272.4 Hz, C_{12}), δ 128.6 (C_7 or C_8) δ 128.8 (C_7 or C_8), δ 130.7 (q, $^2J_{CF}$ = 32.1 Hz, C_5), δ 142.4 (C_3).

^{19}F {^1H} NMR/$CDCl_3$: δ -61.98.

HRMS: calculated for $C_{10}H_{10}F_3O_2$ (ES^- MS): 219.0633. Found: 219.0630.

Elemental analysis: Calculated: C: 54.55%; H: 5.04%; F: 25.88%. Found: C: 53.57%; H: 5.03%; F: 25.68%.

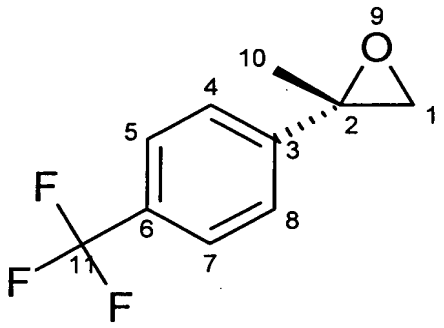
$[\alpha]_D^{22}$ = -5.9 (c 1.25; $CHCl_3$) [(*S*)/ee = 59.0%].

Example 28

The procedure described in example 23 is applied to methyl-(4-trifluoromethylphenyl) oxirane. (*S*)-methyl-(4-trifluoromethylphenyl) oxirane (yield = 35.0%; ee = 99.1%) and (*R*)-methyl-(4-trifluoromethylphenyl)ethane-1,2-diol (yield = 58.0%; ee = 88.3%) are obtained.

Structural analysis

(*S*)-methyl-(4-trifluoromethylphenyl) oxirane



^1H NMR/$CDCl_3$: δ 1.73 (s, 3H, H_{10}), δ 2.76 (d, 1H, $^1J_{HH}$ = 5.4 Hz, H_1), δ 3.00 (d, 1H, $^1J_{HH}$ = 5.4 Hz, H_1), δ 7.48 (d, 2H, $^3J_{HH}$ = 8.1 Hz, $H_{4,8}$), δ 7.59 (d, 2H, $^3J_{HH}$ = 8.1 Hz, $H_{5,7}$).

^{13}C NMR/$CDCl_3$: δ 22.2 (C_{10}), δ 57.1 (C_1), δ 57.8 (C_2), δ 124.9 (q, $^1J_{CF}$ = 270.3 Hz, C_{11}), δ 126.1 (q, $^3J_{CF}$ = 3.8 Hz, C_5 and

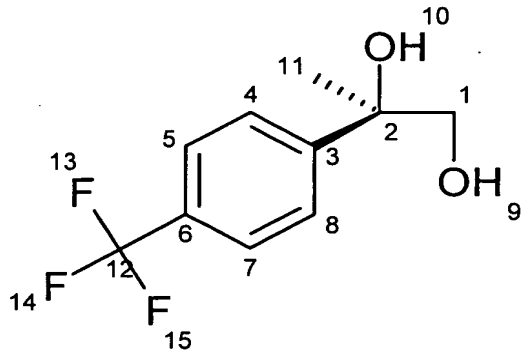
C_7), δ 126.5 (C_4 and C_8), δ 130.5 (q, $^2J_{CF}$ = 31.9 Hz, C_6), δ 146.1 (C_3).

^{19}F {^1H} NMR/$CDCl_3$: δ -62.13.

HRMS: calculated for $C_{10}H_8F_3O$ (ES^- MS): 201.0527. Found: 201.0536.

[α]$_D^{22}$ = +16.7 (c 0.89; $CHCl_3$) [(*S*)/ee = 99.1%].

(*R*)-methyl-(4-trifluoromethylphenyl)-ethane-1,2-diol



Mp: 59°C (Lit.: 57.5°C according to Suprun, W.; *J. Prakt. Chem* **1996**, *338*, 231-237. "Untersuchungen zur oxidation von p-substituierten alpha-methylstyrolen".

^1H NMR/$CDCl_3$: δ 1.55 (s, 3H, H_{11}), δ 1.84 (dd, 1H, $^3J_{H9H1}$ = 5 Hz, $^3J_{H9H1'}$ = 7.25 Hz, H_9), δ 2.68 (s, 1H, H_{10}), δ 3.74 (ddd, 2H, $^1J_{H1H1'}$ = 11 Hz, $^3J_{H9H1}$ = 5 Hz, $^3J_{H9H1'}$ = 7.25 Hz, $H_{1,1'}$), δ 7.58 (d, 2H, $^3J_{HH}$ = 8.75 Hz, $H_{4,8}$), δ 7.63 (d, 2H, $^3J_{HH}$ = 8.75 Hz, $H_{5,7}$).

^{13}C NMR/Acetone-d_6: δ 26.4 (C_{11}), δ 71.5 (C_1), δ 75.0 (C_2), δ 125.4 (q, $^3J_{CF}$ = 3.8 Hz, C_5 and C_7), δ 125.6 (q, $^1J_{CF}$ = 269.6 Hz, C_{12}), δ 127.1 (C_4 and C_8), δ 128.3 (q, $^2J_{CF}$ = 32.1 Hz, C_6), δ 152.6 (C_3).

^{19}F {^1H} NMR/$CDCl_3$: δ -61.98.

HRMS: calculated for $C_{10}H_{10}F_3O_2$ (ES^- MS): 219.0633. Found: 219.0614.

[α]$_D^{22}$ = -9.4 (c 1.03; $CHCl_3$) [(*S*)/ee = 88.3%].

Example 29

645 mg of racemic 4-(trifluoromethylthiophenyl) oxirane (i.e. 2.93 mmol) are placed in 6.45 ml of isooctane, in a

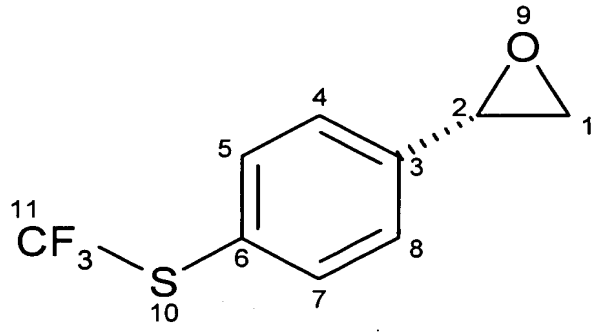
flask equipped with mechanical stirring. 57.05 ml of distilled water are added. The entire mixture is placed in a bath thermostated at 27°C, with stirring. During this time, an enzymatic solution containing 12.9 mg per ml of water of recombinant *An* EH enzymatic extract (purity 25%) is prepared. This solution is itself also placed at 27°C. After 30 minutes, 1 ml of the enzymatic solution previously prepared is added to the reaction medium. This moment corresponds to time t_0.

The selected monitoring and treatment of the reaction are those presented in example 23.

(*S*)-4-(trifluoromethylthiophenyl) oxirane (yield = 37.2%; ee = 98.0%) and (R)-4-(trifluoromethylthiophenyl)ethane-1,2-diol (yield = 38.9%; ee = 85.0%) are obtained.

Structural analysis

<u>(*S*)-4-(trifluoromethylthiophenyl) oxirane</u>



^{1}H NMR/CDCl$_3$: δ 2.78 (dd, 1H, $^3J_{HH}$ = 2.5 Hz, $^1J_{HH}$ = 5.5 Hz, H_1), δ 3.18 (dd, 1H, $^3J_{HH}$ = 4.0 Hz, $^1J_{HH}$ = 5.5 Hz, H_1), δ 3.89 (dd, 1H, $^3J_{HH}$ = 2.5 Hz, $^3J_{HH}$ = 4.0 Hz, H_2), δ 7.37 (d, 2H, $^3J_{HH}$ = 8.2 Hz, $H_{4,8}$), δ 7.64 (d, 2H, $^3J_{HH}$ = 8.2 Hz, $H_{5,7}$).

^{13}C NMR/DMSO-d$_6$: δ 66.9 (C_1), δ 73.1 (C_2), δ 124.0 (q, J = 1.9 Hz, C_6), δ 127.9 (C_4 and C_8), δ 129.6 (q, $^1J_{CF}$ = 307.6 Hz, C_{11}), δ 135.7 (C_5 and C_7), δ 147.4 (C_3).

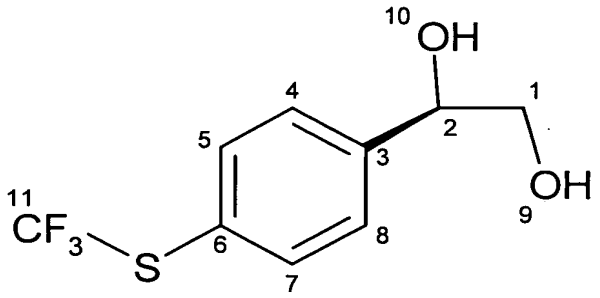
^{19}F {^{1}H} NMR/CDCl$_3$: δ -43.27.

HRMS: calculated for $C_9H_6F_3OS$ (ES^- MS): 219.0091. Found: 219.0100.

Elemental analysis: Calculated: C: 49.09%; H: 3.20%; F: 25.88%. S: 14.56%. Found: C: 49.41%; H: 3.22%; F: 27.11%; S: 13.38%.

$[\alpha]_D^{22}$ = +21.1 (c 1.20; $CHCl_3$) [(*S*)/ee = 98.5%].

(*R*)-4-(trifluoromethylthiophenyl)ethane-1,2-diol



Mp: 72°C.

^{1}H NMR/DMSO-d_6: δ 3.46 (m, 2H, $H_{1,1'}$), δ 4.61 (dd, 1H, J = 4.25 Hz, J = 5.75 Hz, H_2), δ 4.83 (dd, 1H, J = 5.75 Hz, J = 5.75 Hz, H_9), δ 5.47 (d, 1H, J = 4.25 Hz, H_{10}), δ 7.51 (d, 2H, $^3J_{HH}$ = 8Hz, $H_{4,8}$), δ 7.67 (d, 2H, $^3J_{HH}$ = 8 Hz, $H_{5,7}$).

^{13}C NMR/DMSO-d_6: δ 67.9 (C_1), δ 74.7 (C_2), δ 120.8 (m, C_6), δ 130.0 (q, $^1J_{CF}$ = 318.4 Hz, C_{11}), δ 127.9 (C_4 and C_8), δ 135.8 (C_5 and C_7), δ 147.4 (C_3).

^{19}F {^{1}H} NMR/$CDCl_3$: δ -42.22.

HRMS: calculated for $C_9H_8F_3O_2S$ (ES^- MS): 237.0197. Found: 237.0190.

Elemental analysis: Calculated: C: 48.35%; H: 3.81%; F: 23.92%. S: 13.46%. Found: C: 46.04%; H: 3.68%; F: 24.62%; S: 13.69%.

$[\alpha]_D^{22}$ = -5 (c 1.18; $CHCl_3$) [(*S*)/ee = 85.0%].

Determination of the absolute configurations

Diols not described in the literature

We applied a method using circular dichroism described on substrates of the same type, by Bar, L.D.; Pescitelli, G.; Pratelli, C.; Pini, D.; Salvadori, P., Determination of absolute configuration of acyclic 1,2-diols with $Mo_2(OAc)_4$. Snatzke's method revisited, *J. Org. Chem.*, **2001**, *66*, 4819-4825.

Experimental protocol

An amount of diol is added to a solution of approximately 0.6 to 0.7 $mg.ml^{-1}$ of commercial $Mo_2(AcO)_4$ in DMSO such that the ligand/metal ratio is between 0.6:1.2 (for substrates of low optical purity). The first ICD (Induced Circular Dichroism) is measured immediately after mixing and a verification is carried out every ten minutes until stabilization (40-50 min).

Results

Diol of the example	ee	Concentration (mM)	Diol/ $Mo_2(OAc)_4$ ratio	ICD bands, λ_{ext} (nm), $\Delta\varepsilon_{ext}$			
				V	IV	III	II
24	77	0.375	1	280 (0.12)	305 (-0.66)	348 (0.06)	385 (-0.18)
25	13.2	0.75	1	279 (0.04)	301 (-0.14)	352 (-0.01)	375 (-0.05)
27	59	0.75	1	277 (0.15)	316 (-0.22)	342 (-0.01)	385 (-0.05)
26	84.3	0.375	1	270 (0.07)	308 (-0.60)	350 (-0.12)	381 (-0.20)
28	88.3	0.375	1	273 (0.15)	308 (-0.18)	350 (0.02)	379 (-0.06)
29	85	0.375	1	271 (0.22)	308 (-1.12)	352 (-0.18)	379 (0.30)
23	94.5	0.375	1	272 (0.26)	310 (-1.22)	352 (-0.22)	375 (-0.33)

Circular dichroism; experimental conditions and results ($\Delta\varepsilon$ normalized with respect to the diol concentration)

According to the rules described by Snatzke, all the diols tested are of absolute configuration (R).

Epoxides not described in the literature

The absolute configuration of the epoxides is obtained by chemical correlation. The diols are cyclized to epoxide with retention of configuration (method described below in example 30). Injection into chiral GC makes it possible, by comparison of the chromatogram obtained with the epoxide derived from the enzymatic reaction, to deduce the absolute configuration of the epoxide.

Example 30

Cyclization of (*R*)-methyl-(3-trifluoromethylphenyl)ethane-1,2-diol

11.0 mg (1 mmol) of (R)-methyl-(3-trifluoromethyl-phenyl)ethane-1,2-diol (ee = 88.3%) and 500 µl of THF are placed in a 3 ml conical minireactor equipped with magnetic agitation. One equivalent of tosyl chloride in solution in 500 µl of THF is added. After agitation at ambient temperature for one hour, 6 equivalents of sodium hydride are added. After agitation for 12 h, 100 µl of water are added and extraction is carried out with 1 ml of ethyl ether. The organic phase is injected into chiral CG.

This technique made it possible to show that all the residue epoxides described in examples 23 to 29 were of absolute configuration (S).

Example 31

Analytical scale

The procedure described in example 1 is applied to 2-(4-trifluoromethylphenoxymethyl) oxirane (6.54 mg, i.e. [2-(4-trifluoromethylphenoxymethyl) oxirane] = 3 mM), in the presence of 30% of DMSO. The enzymatic solution added (100 µl) contains 2 mg of enzymatic extract of the recombinant *An* EH (characterized as having a purity of the order of 25%) in 4.434 ml of distilled water. The internal standard is 3-bromoacetophenone.
The values obtained correspond to an apparent enantioselectivity coefficient E of 7.

Analyses carried out on a column of Chirasil-Dex CB type (T = 110°C; tr = 33.2 min and 33.8 min for the two enantiomers of the epoxide; tr = 22.2 for the internal standard).

Preparatory scale

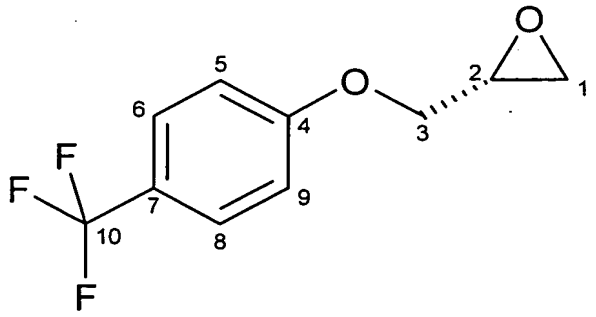
763 mg of 2-(4-trifluoromethylphenoxymethyl) oxirane (i.e. 3.5 mmol) in 105 ml of DMSO are placed in a 1 l fermenter. 664 ml of distilled water are added. The entire mixture is subjected to stirring, the temperature being controlled and maintained at 27°C. An enzymatic solution containing 0.763 mg of recombinant An enzymatic extract (purity 25%) per ml of water is prepared. After 30 minutes, 1 ml of the enzymatic solution previously prepared is added to the reaction medium. This moment corresponds to time t_0.

The reaction is monitored by chiral GC. A 150 µl sample of reaction medium is regularly taken and added to 100 µl of acetonitrile and 100 µl of isooctane, placed beforehand in an eppendorf. After vortexing and centrifugation, the mixture is injected into chiral GC in order to measure the enantiomeric excess of the residue epoxide. When the enantiomeric excess of the residue epoxide reaches the value of 80%, the reaction is stopped with adding 150 ml of ethyl acetate. The mixture is allowed to separate by settling out, the organic phase is recovered and the aqueous phase is extracted with 2 × 50 ml of ethyl

acetate. The aqueous phases are combined, washed with 200 ml of saturated aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The residue epoxide and the diols formed are separated by flash chromatography on silica gel (50 parts; eluent: 90/10 hexane/ethyl acetate up to pure ethyl acetate). With a view to measuring the optical rotations, each isolated product is subsequently subjected to bulb-to-bulb purification so as to eliminate all traces of solvent and of silica. 369 mg of (*S*)-2-(4-trifluoromethylphenoxymethyl)ethane-1,2-diol (yield = 44.7%; ee = 85.4%) and 194 mg of (*R*)-2-(4-trifluoromethylphenoxymethyl) oxirane (yield = 25.4%; ee = 79.4%) are obtained.

Structural analysis

(*R*)-2-(4-trifluoromethylphenoxymethyl) oxirane



^{1}H NMR/$CDCl_3$:

δ 2.69 (dd, 1H, J = 2.5 Hz, J = 4.75 Hz, H_1), δ 2.84 (dd, 1H, J = 4.75 Hz, J = 4.25 Hz, H_1), δ 3.3 (m, 1H, H_2), δ 3.98 (dd, 1H, J = 6 Hz, J = 11 Hz, H_3), δ 4.22 (dd, 1H, J = 3 Hz, J = 11 Hz, H_3), δ 7.18 (m, A_2B_2, 4H, aromatic protons).

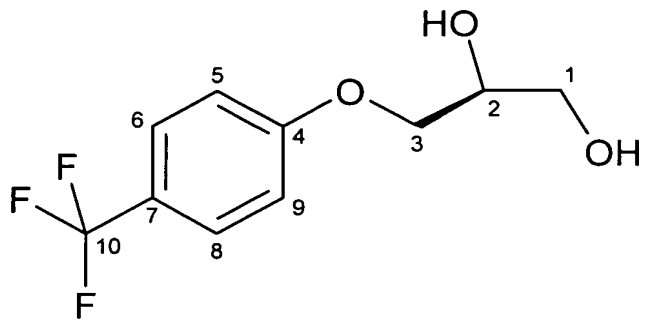
^{13}C NMR/$CDCl_3$:

δ 44.2 (C_1), δ 49.6 (C_2), δ 68.6 (C_3) δ 114.3 (C_5 and C_9), δ 123.1 (q, $^2J_{CF}$ = 32.4 Hz, C_7), δ 124.1 (q, $^1J_{CF}$ = 269.6 Hz, C_{10}), δ 126.6 (q, $^3J_{CF}$ = 3.5 Hz, C_6 and C_8), δ 160.6 (C_4).

^{19}F {^{1}H} NMR/$CDCl_3$: δ -61.69.

$[\alpha]_D^{22}$ = +4.5 (c 1.0; $CHCl_3$) [(*S*)/ee = 79.4%].

(*S*)-2-(4-trifluoromethylphenoxymethyl)ethane-1,2-diol



^{1}H NMR/CD_3OD:

δ 3.21 (dd, 1H, J = 1.5 Hz, J = 3.25 Hz), δ 3.57 (m, 2H), δ 3.95 (m, 3H), δ 7.24 (m A_2B_2, 4H, aromatic protons).

^{13}C NMR/CD_3OD:

δ 62.3 (C_1), δ 68.9 (C_2), δ 69.9 (C_3), δ 114.1 (C_5 and C_9), δ 122.1 (q, $^2J_{CF}$ = 32.2 Hz, C_7), δ 124.2 (q, $^1J_{CF}$ = 286,1 Hz, C_{10}), δ 126.2 (q, $^3J_{CF}$ = 3.8 Hz, C_6 and C_8), δ 161.4 (C_4).

$[\alpha]_D^{22}$ = -6.6(c 1.1; $CHCl_3$) [(*S*)/ee = 85.4%].

It should be clearly understood that the invention defined by the attached claims is not limited to the specific embodiments indicated in the above description, but encompasses the variants thereof that depart neither from the scope nor from the spirit of the present invention.